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	L11	Salin-Nordstrom-T-H.IN.	1
	L10	(Salin-Nordstrom-Tuija-H.IN.)	0
	L9	(L7 AND L8)	25
	L8	transdifferentiation	171
	L7	astrocyte	4463
	L6	L5 AND transdifferentiation	17
	L5	L4 AND astrocyte	1413
	L4	435/325,363,366,368.CCLS.	16676
	L3	Salin-Nordstrom-T.IN.	0
	L2	Salin-Nordstrom.IN.	1
	L1	(Salin-Nordstrom-Tuija.IN.)	0

END OF SEARCH HISTORY

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s astrocyte
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.1
       168587 ASTROCYTE
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59 FILES SEARCHED...
        25041 TRANSDIFFERENTIATION
> S L1 AND L2
60 FILES SEARCHED..
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ROCESSING COMPLETED FOR L3
            55 DUP REM L3 (55 DUPLICATES REMOVED)
> D L4 1-55
.4
    ANSWER 1 OF 55 USPATFULL on STN
      2004:150954 USPATFULL
١N
      Methods for treating disorders of neuronal deficiency with bone
Ί
      marrow-derived cells
      Blau, Helen M., Menlo Park, CA, UNITED STATES
      Brazelton, Timothy, Cupertino, CA, UNITED STATES
      Weimann, James M., Palo Alto, CA, UNITED STATES
The Board of Trustees of the Leland, Palo Alto, CA (U.S. corporation)
Α
ľ
      US 2004115175
                         Α1
                                20040617
      US 2003-688747
I
                          Α1
                                20031016 (10)
      Continuation-in-part of Ser. No. US 2001-993045, filed on 13 Nov 2001,
LI
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RAI
      US 2000-247128P
                           20001110 (60)
      Utility
Т
S
      APPLICATION
N.CNT 2455
NCL
      INCLM: 424/093.700
      NCLM: 424/093.700
CL
C
      [7]
      ICM: A61K045-00
4
    ANSWER 2 OF 55 USPATFULL on STN
      2004:140277 USPATFULL
Ν
Ι
      Multipotent adult stem cells, sources thereof, methods of obtaining
      same, methods of differentiation thereof, methods of use thereof and
      cells derived thereof
Ν
      Furcht, Leo T, Minneapolis, MN, UNITED STATES
      Verfaillie, catherine M, St Paul, MN, UNITED STATES
Reyes, Morayma, Minneapolis, MN, UNITED STATES
      US 2004107453
Ι
                               20040603
                          Α1
      US 2004-467963
I
                          Α1
                                20040105 (10)
      WO 2002-US4652
                                20020214
Т
      Utility
S
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N.CNT 4100
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      INCLM: 800/018.000
      INCLS: 424/093.700; 800/021.000; 435/353.000; 435/354.000; 435/366.000
CL
      NCLM:
             800/018.000
      NCLS:
             424/093.700; 800/021.000; 435/353.000; 435/354.000; 435/366.000
C
      ICM: A01K067-027
      ICS: C12N005-06: C12N005-08
AS INDEXING IS AVAILABLE FOR THIS PATENT.
   ANSWER 3 OF 55 USPATFULL ON STN
      2004:113656 USPATFULL
Ι
      Immune privileged cells for delivery of proteins and peptides
      John, Constance Mary, San Francisco, CA, UNITED STATES
      US 2004086494
Т
                               20040506
                          Α1
      US 2001-941398
                          Α1
                               20010828 (9)
      Continuation-in-part of Ser. No. US 1998-131501, filed on 9 Aug 1998,
LI
      ABANDONED Continuation-in-part of Ser. No. US 1996-726531, filed on 7
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Oct 1996, ABANDONED
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        NCLS:
                435/366.000
ΙC
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        ICM: A61K048-00
        ICS: C12N005-08
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
     ANSWER 4 OF 55 USPATFULL ON STN
١N
        2004:82751 USPATFULL
ΓΙ
       Neurogenesis from hepatic stem cells
       Petersen, Bryon E., Gainesville, FL, UNITED STATES Deng, Jie, Gainesville, FL, UNITED STATES
[N
PΙ
       US 2004063202
                                       20040401
                                Α1
       US 2003-651829
                                       20030828 (10)
l
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       US 2002-406513P
PRAI
                                 20020828 (60)
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T
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CAS INDEXING IS AVAILABLE FOR THIS PATENT.
     ANSWER 5 OF 55 USPATFULL ON STN
       2004:30644 USPATFULL
١N
       Proteins and nucleic acids encoding same
       Spytek, Kimberly A., New Haven, CT, UNITED STATES
       Li, Li, Branford, CT, UNITED STATES
       Wolenc, Adam R., New Haven, CT, UNITED STATES
       Vernet, Corine, North Branford, CT, UNITED STATES
       Eisen, Andrew J., Rockville, MD, UNITED STATES
       Liu, Xiaohong, Lexington, MA, UNITED STATES
       Malyankar, Uriel M., Branford, CT, UNITED STATES
       Shimkets, Richard A., Guilford, CT, UNITED STATES Tchernev, Velizar, Branford, CT, UNITED STATES Spaderna, Steven K., Berlin, CT, UNITED STATES Gorman, Linda, Branford, CT, UNITED STATES
       Kekuda, Ramesh, Norwalk, CT, UNITED STATES
       Patturajan, Meera, Branford, CT, UNITED STATES
       Gusev, Vladimir Y., Madison, CT, UNITED STATES
       Gangolli, Esha A., Madison, CT, UNITED STATES
       Guo, Xiaojia (Sasha), Branford, CT, UNITED STATES
       Shenoy, Suresh G., Branford, CT, UNITED STATES
Rastelli, Luca, Guilford, CT, UNITED STATES
Casman, Stacie J., North Haven, CT, UNITED STATES
Boldog, Ferenc L., North Haven, CT, UNITED STATES
Burgess, Catherine E., Wethersfield, CT, UNITED STATES
Edinger, Shlomit R., New Haven, CT, UNITED STATES
Filerman Karen Branford CT UNITED STATES
       Ellerman, Karen, Branford, CT, UNITED STATES
       Gunther, Erik, Branford, CT, UNITED STATES
Smithson, Glennda, Guilford, CT, UNITED STATES
       Millet, Isabelle, Milford, CT, UNITED STATES
       MacDougall, John R., Hamden, CT, UNITED STATES
       US 2004022781
                                      20040205
                               Α1
       US 2001-38854
                                Α1
                                      20011231 (10)
       US 2000-258928P
                                 20001229 (60)
RAI
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                                 20010102 (60)
                                 20010104 (60)
       US 2001-259785P
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       US 2001-279832P
                                 20010329 (60)
       US 2001-279833P
                                 20010329 (60)
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                                 20010329 (60)
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       US 2001-283889P
       US 2001-284447P
                                 20010418
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       US 2001-286683P
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       US 2001-313325P
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US 2001-333350P
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        ICM: C12Q001-68
        ICS: G01N033-53; G01N033-567; C07H021-04; A61K039-395; C12P021-02;
        C12N005-06; C07K014-47; C07K016-22
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     ANSWER 6 OF 55 USPATFULL on STN 2004:41477 USPATFULL Laminin 5, 13 and 14 and uses thereof
L4
ΑN
ΤI
       Brunken, William J., Canton, MA, United States
Libby, Richard R., Hingham, MA, United States
ΙN
       Hunter, Dale D., Canton, MA, United States
       Burgeson, Robert E., Marblehead, MA, United States
The General Hospital Corporation, Boston, MA, United States (U.S.
PA
        corporation)
PΙ
       us 6693169
                                    20040217
                              В1
ΑI
       US 1999-415625
                                    19991012 (9)
                               19981015 (60)
PRAI
       US 1998-104430P
       US 1998-104044P
                               19981013 (60)
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FS
       GRANTED
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INCL
       INCLM: 530/350.000
       INCLS: 530/362.000
NCL
       NCLM:
               530/350.000
       NCLS:
               530/362.000
IC
        [7]
       ICM: C07K014-78
EXF
        435/320.1; 435/252.3; 435/365.1; 514/8; 536/23.1; 530/350; 530/362
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
L4
     ANSWER 7 OF 55 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 1
٩N
     2004:361456
                    CAPLUS
ΤI
                      ***transdifferentiation***
     Bone marrow
                                                        in brain after transplantation:
     a retrospective study
     Cogle, Christopher R.; Yachnis, Anthony T.; Laywell, Eric D.; Zander, Dani S.; Wingard, John R.; Steindler, Dennis A.; Scott, Edward W.
٩U
CS
     Program in Stem Cell Biology and Regenerative Medicine, University of
     Florida Shands Cancer Center, Gainesville, FL, USA
     Lancet (2004), 363(9419), 1432-1437
CODEN: LANCAO; ISSN: 0140-6736
SO
PB
     Elsevier Science Ltd.
TC
     Journal
     English
LA
RE.CNT
        <sup>-</sup>39
                THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD
                ALL CITATIONS AVAILABLE IN THE RE FORMAT
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     ANSWER 8 OF 55 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
٩N
     2004:374735
                    SCISEARCH
GΑ
     The Genuine Article (R) Number: 812RW
     Umbilical cord blood stem cells can expand hematopoietic and neuroglial
ΓI
     progenitors in vitro
     McGuckin C P (Reprint); Forraz N; Allouard Q; Pettengell R
St George Hosp, Sch Med, King George Lab, Cranmer Terrace, London SW17
٩U
CS
     ORE, England (Reprint); St George Hosp, Sch Med, King George Lab, London
     SW17 ORE, England; Kingston Univ, London, England; Kingston Univ, Sch Life
     Sci, Kingston upon Thames KT1 2EE, Surrey, England; St George Hosp, Sch
     Med, Dept Basic Med Sci, London, England; St George Hosp, Sch Med, Dept
     Cellular & Mol Med, London, England
CYA
     England
50
     EXPERIMENTAL CELL RESEARCH, (1 MAY 2004) Vol. 295, No. 2, pp. 350-359.
     Publisher: ACADEMIC PRESS INC ELSEVIER SCIENCE, 525 B ST, STE 1900, SAN
     DIEGO, CA 92101-4495 USA.
     ISSN: 0014-4827.
     Article: Journal
     English
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REC
     Reference Count: 57
     "ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS"
     ANSWER 9 OF 55 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
L4
ΑN
     2004:300585
                    BIOSIS
     PREV200400301616
DN
     Fate of donor hematopoietic cells in demyelinating mutant mouse, twitcher,
ΤI
     following transplantation of GFP+ bone marrow cells.
     Yagi, Takashi; McMahon, Eileen J.; Takikita, Shoichi; Mohri, Ikuko; Matsushima, Glenn K.; Suzuki, Kinuko [Reprint Author]
Dept Pathol and Lab Med, Univ N Carolina, 919A Brinkhous Bullitt
ΑU
CS
     Bldg,CB 7525, Chapel Hill, NC, 27599, USA
     kis@med.unc.edu
     Neurobiology of Disease, (June 2004) Vol. 16, No. 1, pp. 98-109. print.
SO
     ISSN: 0969-9961 (ISSN print).
DT
     Article
     English
1 A
FD
     Entered STN: 30 Jun 2004
     Last Updated on STN: 30 Jun 2004
L4
     ANSWER 10 OF 55 IFIPAT COPYRIGHT 2004 IFI on STN DUPLICATE 3
      10315526 IFIPAT; IFIUDB; IFICDB
ΑN
TI
      TRANS-DIFFERENTIATION AND RE-DIFFERENTIATION OF SOMATIC CELLS AND
      PRODUCTION OF CELLS FOR CELL THERAPIES; CONTROLLING DIFFERENTIATION IN
      SOMATIC CELLS; GENERATE PREFERENTIAL SOMATIC CELL CULTURE, INCUBATE WITH
      ENZYME INHIBITORS, MONITOR DIFFERENTATION INTO ALTERNATE CELL TYPE
      Dominko Tanja; Malcuit Christopher; Page Raymond
IN
      Unassigned Or Assigned To Individual (68000)
PA
PΙ
      us 2003059939
                        A1
                             20030327
      us 2002-228296
                             20020827
ΑI
      US 2001-314654P
                             20010827 (Provisional)
PRAI
                             20030327
      US 2003059939
FI
DT
      Utility; Patent Application - First Publication
FS
      CHEMICAL
      APPLICATION
CLMN
      20
        5 Figure(s).
GΙ
     FIGS. I and 2: Cells with neuronal morphology produced by treating bovine
      fetal fibroblasts CB at 2.5-7.5 mu g/m and culturing them under
       conditions that induce neural differentiation. The cells in FIG. 1 were
      observed with phase contrast microscopy; those in FIG. 2 were observed by DIC. FIG. 1: (A) Control, (B) 2.5 mu g/ml, (C) 5.0, mu g/ml, (D) 7.5 mu
      g/ml
     FIGS. 3 and 4: Cells with neuronal morphology produced by treating bovine
       adult fibroblasts CB at 10.0 mu \mathrm{g/m} and culturing them under conditions
       that induce neural differentiation.
     FIG. 5: Cells with neuronal morphology produced by treating human fetal
       fibroblasts CB at 5.0 mu g/m and culturing them under conditions that
       induce neural differentiation.
      (A) Control, (B) 2.5 mu g/ml, (C) 5.0 mu g/ml, (D) 7.5 mu g/ml
     FIG. 6: Photographs showing the presence of neural-specific markers nestin and Tujl in human fetal fibroblasts treated with CB at 5.0 mu g/m and
      cultured under conditions that induce neural differentiation.
L4
     ANSWER 11 OF 55 USPATFULL ON STN
        2003:312269
                     USPATFULL
ΑN
        Stem cell-like cells
TI
        Kruijer, Wiebe, Leusden, NETHERLANDS
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        us 2003219866
                                  20031127
PΤ
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        us 2003-349505
                             Α1
                                  20030121 (10)
ΑI
        Continuation of Ser. No. WO 2001-NL561, filed on 20 Jul 2001, UNKNOWN
RLI
                              20000721
        EP 2000-202634
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DT
        APPLICATION
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INCL
        INCLM: 435/069.100
        INCLS: 435/320.100; 435/366.000; 530/350.000; 536/023.500
NCL
        NCLM:
               435/069.100
        NCLS:
               435/320.100; 435/366.000; 530/350.000; 536/023.500
IC
        ICM: C07K014-475
        ICS: C07H021-04; C12P021-02; C12N005-08
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
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L4

ANSWER 12 OF 55 USPATFULL ON STN

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2003:276702 USPATFULL
       Phenotypic screen of chimeric proteins
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       Seol, Wongi, Yuseong-gu, KOREA, REPUBLIC OF
       Lee, Horim, Chungcheongnam-do, KOREA, REPUBLIC OF
       Lee, Seong-Il, Yuseong-gu, KOREA, REPUBLIC OF
       Yang, Hyo-Young, Yuseong-gu, KORÉA, REPUBLIC OF
Lee, Yangsoon, Yuseong-gu, KOREA, REPUBLIC OF
      Jang, Young-Soon, Yuseong-gu, KOREA, REPUBLIC OF US 2003194727 A1 20031016
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N.CNT
      5577
       INCLM: 435/006.000
NCL
       INCLS: 435/069.100; 435/320.100; 435/325.000; 435/252.300; 435/007.200;
               435/254.200; 435/219.000
               435/006.000
ICL
       NCLM:
       NCLS:
              435/069.100; 435/320.100; 435/325.000; 435/252.300; 435/007.200;
               435/254.200; 435/219.000
       [7]
C
       ICM: C12Q001-68
       ICS: G01N033-53; G01N033-567; C12N001-18; C12P021-02; C12N001-21;
       C12N005-06
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INCL
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       NCLM:
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       diabetes mellitus
      Habener, Joel F., Newton Centre, MA, UNITED STATES Zulewski, Henryk, Basel, SWITZERLAND
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       Thomas, Melissa K., Boston, MA, UNITED STATES
       Abraham, Elizabeth J., Quincy, MA, UNITED STATES Vallejo, Mario, Madrid, SPAIN
       Leech, Colin A., Boston, MA, UNITED STATES
       Nolan, Anna Louise, Brookline, MA, UNITED STATES
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INCL
       INCLS: 435/366.000
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ICL
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       ICM: A61K048-00
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      Weindruch, Richard H., Madison, WI, UNITED STATES
Prolla, Tomas A., Madison, WI, UNITED STATES
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      Lee, Cheol-Koo, Madison, WI, UNITED STATES
Kayo, Tsuyoshi, Madison, WI, UNITED STATES
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N.CNT 2422
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      INCLS: 435/007.210
NCLM: 435/006.000
CL
       NCLS:
               435/007.210
C
       ICM: C12Q001-68
       ICS: G01N033-567
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       Habener, Joel F., Newton Centre, MA, UNITED STATES Zulewski, Henryk, Basel, SWITZERLAND
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       Faustman, Denise L., Weston, MA, UNITED STATES
      Thomas, Melissa K., Boston, MA, UNITED STATES
Massachusetts General Hospital (U.S. corporation)
Ι
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S
N.CNT 2495
NCL
       INCLM: 424/093.210
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       NCLS: 424/093.700
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       Lu, Kuanghui, Brookline, MA, United States
       Pang, Kevin, Canton, MA, United States
      Rubin, Lee, Wellesley, MA, United States
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N.CNT 3624
NCL
       INCLM: 435/325.000
       INCLS: 435/363.000; 435/366.000; 435/372.200; 435/375.000; 435/377.000;
               435/384.000; 435/387.000; 435/391.000; 435/392.000
\mathsf{CL}
       NCLM:
               435/325.000
               435/363.000; 435/366.000; 435/372.200; 435/375.000; 435/377.000;
       NCLS:
               435/384.000; 435/387.000; 435/391.000; 435/392.000
C
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ICS: C12N005-08

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ICM: C12N005-00
       ICS: C12N005-06
       435/325; 435/363; 435/366; 435/372.2; 435/375; 435/377; 435/384;
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       435/387; 435/391; 435/392
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    The Genuine Article (R) Number: 674HH
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    Toyoake, Aichi 4701192, Japan (Reprint); Nagoya City Univ, Grad Sch
    Pharmaceut Sci, Dept Mol Hlth Sci, Nagoya, Aichi, Japan
AY:
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    JOURNAL OF NEUROSCIENCE RESEARCH, (15 MAY 2003) Vol. 72, No. 4, pp.
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    Article; Journal
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   Reference Count: 17
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    Kubis N (Reprint); Catala M
Univ Paris 06, Lab Histol & Embryol, 105 Blvd Hop, F-75634 Paris 13,
France (Reprint); Univ Paris 06, Lab Histol & Embryol, F-75634 Paris 13,
France; Univ Paris 06, UMR CNRS 7000, Fac Med Pitie Salperiere, F-75634
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    Paris, France; Hop Lariboisiere, Lab Explorat Fonctionnelles Syst Nerveux
    Pr Levy, F-75475 Paris, France
YA
   France
    NEUROCHIRURGIE, (SEP 2003) Vol. 49, No. 4, pp. 449-456.
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    precursor functions
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    Munoz-Elias G; Woodbury D; Black I B (Reprint)
    UMDNJ, Robert Wood Johnson Med Sch, Dept Neurosci & Cell Biol, 675 Hoes
S
    Lane, CABM Bldg, Room 342, Piscataway, NJ 08854 USA (Reprint); UMDNJ,
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      ***Transdifferentiation***
                                       potential of human hematopoietic cells
    isolated from mobilized peripheral blood.
   Kuci, Selim [Reprint Author]; Schilbach, Karin [Reprint Author]; Handgretinger, Rupert; Buehring, Hans-Joerg [Reprint Author]; Jurecic, Roland; Schumm, Michael [Reprint Author]; Lang, Peter [Reprint Author];
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San Diego, CA, USA. December 06-09, 2003. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971.
Conference; (Meeting)
Conference; (Meeting Poster)
Conference; Abstract; (Meeting Abstract)
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Entered STN: 10 Mar 2004
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   ***Astrocytes***
                       as stem cells: Nomenclature, phenotype, and
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 Steindler D.A.; Laywell E.D.
 Dr. D.A. Steindler, Department of Neuroscience, McKnight Brain Institute,
 University of Florida, 100 S. Newell Drive, Gainesville, FL 32610, United
 States.
 E-mail: steindler@mbi.ufl.edu
 GLIA, (01 JUL 2003), 43/1 (62-69), 77 reference(s)
 CODEN: GLIAEJ ISSN: 0894-1491
 Journal: Article
 United States
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Greco B (Reprint); Recht L
Univ Massachusetts, Sch Med, Dept Neurol, 55 Lake Ave N, Worcester, MA
01655 USA (Reprint); Univ Massachusetts, Sch Med, Dept Neurol, Worcester,
MA 01655 USA
USA
JOURNAL OF CELLULAR BIOCHEMISTRY, (1 JAN 2003) Vol. 88, No. 1, pp. 51-56.
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 Fetal human hematopoietic stem cells can differentiate sequentially into
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 Hao H.-N.; Zhao J.; Thomas R.L.; Parker G.C.; Lyman W.D. Dr. H.-N. Hao, Department of Pediatrics, Children's Res. Center of
 Michigan, 3901 Beaubien, Detroit, MI 48201, United States.
 E-mail: HsiaoNan.Hao@wayne.edu
 Journal of Hematotherapy and Stem Cell Research, (2003), 12/1 (23-32), 59
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  ***Transdifferentiation***
                                 of adult bone marrow stem cells into neural
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Sogos, V. [Reprint Author]; Reali, C. [Reprint Author]; Scintu, F.
[Reprint Author]; Pillai, R. [Reprint Author]; Badiali, M.; Sanna, A.;
Argiolu, F.
Dept. of Cytomorphology, Univ. of Cagliari, Monserrato (CA), Italy
Society for Neuroscience Abstract Viewer and Itinerary Planner, (2003)
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     Conference: (Meeting)
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     Conference: Abstract: (Meeting Abstract)
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     English
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ΤI
     cells share gene expression profiles and biological properties.
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     Mantegna, L. [Reprint Author]; Fantozzi, R. [Reprint Author]; Silani, V.
     [Reprint Author]
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     Dept. of Neurological Sci., IRCCS Ospedale Maggiore, Milan, Italy
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      DUPLICATE 6
      2003-08715 BIOTECHDS
ΤI
      Preparing neural stem cells, useful for transplantation, e.g. for
      treating neurodegeneration e.g. in multiple sclerosis, by in vitro or in
      vivo culture of hematopoietic stem cells
         stem cell culture for tissue engineering and cell therapy
ΑU
      ALARCON MARTINEZ P; BONILLA JIMENEŽ S; SILVA GONZALEZ A G; MARTINEZ PEREZ
PΑ
      UNIV ELCHE HERNANDEZ MIGUEL
ΡI
     WO 2002096439 5 Dec 2002
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     WO 2002-ES253 27 May 2002
PRAI
      ES 2001-1223 28 May 2001; ES 2001-1223 28 May 2001
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     WPI: 2003-140415 [13]
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AΝ
ΤI
      EMBRYONIC STEM CELLS AND NEURAL PROGENITOR CELLS DERIVED THEREFROM:
      REGENERATION CELLS OF NERVOUS SYSTEM
IN
      Ben-Hur Tamir (IL); Pera Martin Frederick (AU); Reubinoff Benjamin Eithan
      (IL)
PΑ
      Unassigned Or Assigned To Individual (68000)
ΡI
      US 2002164308
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                           20010314 CONTINUATION-IN-PART
PRAI
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     Utility; Patent Application - First Publication
TC
FS
     CHEMICAL
      APPLICATION
CLMN
     73
GΙ
       38 Figure(s).
         1 shows phase contrast micrographs of ES cells and their
     differentiated progeny. A, inner cell mass three days after plating. B,
     colony of ES cells. C, higher magnification of an area of an ES cell colony. D, an area of an ES cell colony undergoing spontaneous
     differentiation during routine passage. E, a colony four days after
     plating in the absence of a feeder cell layer but in the presence of 2000
     units/ml human LIF undergoing differentiation in its periphery, . F,
     neuronal cells in a high density culture. Scale bars: A and C, 25
    microns; B and E, 100 microns; D and F, 50 microns.
FIG. 2 shows marker expression in ES cells and their differentiated
     somatic progeny. A, ES cell colony showing histochemical staining for
     alkaline phosphatase. B. ES cell colony stained with antibody MC-813-70
     recognising the SSEA-4 epitope. C, ES cell colony stained with antibody
     TRAL-60. D, ES cell colony stained with antibody GCTM-2. E, high density
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culture, cell body and processes of a cell stained with antineurofilament 68 kDa protein. F, high density culture, cluster of cells and network of processes emanating from them stained with antibody against neural cell adhesion molecule. G, high density culture, cells showing cytoplasmic filaments stained with antibody to muscle actin. H, high density culture, cell showing cytoplasmic filaments stained with antibody to desmin. Scale bars: A, 100 microns; B-D, and F, 200 microns; E, G and H, 50 microns. FIG. 3 shows RT-PCR analysis of gene expression in ES cells and their differentiated derivatives. All panels show 1.5% agarose gels stained with ethidium bromide. A, expression of Oct-4 and b-actin in ES stem cells and high density cultures. Lane 1, 100 bpDNA ladder. Lane 2, stem cell culture, b-actin. Lane 3, stem cell culture, Oct-4. Lane 4, stem cell culture, PCR for Oct-4 carried out with omission of reverse transcriptase. Lane 5, high density culture, b-actin. Lane 6, high density culture, Oct-4. Lane 7, high density culture, PCR for Oct-4 carried out with omission of reverse transcriptase. b-actin band is 200 bp and Oct-4 band is 320 bp. B, expression of nestin and Pax-6 in neural progenitor cells that were derived from differentiating ES colonies. Left lane, 100 bp DNA ladder; lane 1, b-actin in HX 142 neuroblastoma cell line (positive control for nestin PCR); lane 2, b-actin in neural progenitor cells; lane 3, nestin in HX 142 neuroblastoma cell line; lane 4, nestin in neural progenitor cells; lane 5, nestin PCR on same sample as lane 4 without addition of reverse transcriptase; lane 6, Pax-6; lane 7, Pax-6 PCR on same sample as line 6 without addition of reverse transcriptase. Nestin hand is 20% bp. Bax-6 is 274 bp. 6 expression of transcriptase. Nestin band is 208 bp, Pax-6 is 274 bp. C, expression of glutamic acid decarboxylase in cultures of neurons. Left lane, 100 bp DNA ladder; lane 1, b-actin; lane 2, b-actin PCR on same sample as lane 1 without addition of reverse transcriptase; lane 3, glutamic acid decarboxylase; lane 4 glutamic acid decarboxylase on same sample as lane 3 without addition of reverse transcriptase. Glutamic acid decarboxylase band is 284 bp. D, expression of GABA A alpha 2 receptor. Left lane, 100 bp DNA ladder; lane 1, b-actin; lane 2, GABA A alpha 2 receptor; lane 3, PCR without addition of reverse transcriptase. GABA A alpha 2 receptor subunit band is 471 bp.

FIG. 4 shows histology of differentiated elements found in teratomas formed in the testis of SCID mice following inoculation of HES-1 or HES-2 colonies. A, cartilage and squamous epithelium, HES-2. B, neural rosettes, HES-2. C, ganglion, gland and striated muscle, HES-1. D, bone and cartilage, HES-1. E, glandular epithelium, HES-1. F, ciliated columnar epithelium, HES-1. Scale bars: A-E, 100 microns; F, 50 microns. FIG. 5 shows phase contrast microscopy and immunochemical analysis of

marker expression in neural progenitor cells isolated from differentiating ES cultures. A, phase contrast image of a sphere formed in serum-free medium. B-D, indirect immunofluorescence staining of spheres, 4 hours after plating on adhesive substrate, for N-CAM, nestin, and vimentin respectively. In C and D, cells at the base of the sphere were placed in plane of focus to illustrate filamentous staining; confocal examination revealed that cells throughout the sphere were decorated by both antibodies. Scale bar is 100 microns in all panels.

FIG. 6 shows phase contrast appearance and marker expression in cultures of neurons derived from progenitor cells shown in FIG. 5. A, phase contrast micrograph of differentiated cells emanating from a sphere plated onto adhesive surface. B-H, indirect immunofluorescence microscopy of differentiated cells decorated with antibodies against 200 kDa neruofilament protein (B), 160 kDa neurofilament protein (C), MAP2a+b (D), glutamate (E), synaptophysin (F), glutamic acid decarboxylase (G) and beta-tubulin (H). Scale bars: A, ;B, 100 microns; C, 200mircons; D, 20 microns; E and F, 10 microns; G, 20 microns; H, 25 microns. FIG. 7 shows neural precursors proliferating as a monolayer on a plastic

FIG. 7 shows neural precursors proliferating as a monolayer on a plastic tissue culture dish in the presence of EGF and bFGF. These monolayer cultures of proliferating cells were obtained after prolonged cultivation (2-3 weeks) of the spheres in the presence of growth factors without sub-culturing.

FIG. 8 shows phase contrast appearance of a culture consisting of differentiated neural cells.

FIG. 9 shows phase contrast appearance of a sphere that is formed 72 hours after the transfer of a clump of undifferentiated ES cells into serum free medium (Scale bar 100 microns).

FIG. 10 shows linear correlation between the volume of spheres and the number of progenitor cells within a sphere. Spheres of various diameters that were generated from differentiating ES colonies and were propagated for 14-15 weeks were dissaggregated into single cell suspension and the number of cells per sphere was counted.

FIG. 11 shows indirect immunofluorescence staining of a sphere, 4 hours after plating on adhesive substrate, for N-CAM. The sphere was generated by direct transfer of undifferentiated ES cells into serum free medium

and propagation of the resulting spheres for 5 passages. (Scale bar 100 microns).

FIG. 12 shows indirect immunofluorescence membraneous staining for N-CAM of single cells at the periphery of a sphere 4 hours after plating on adhesive substrate. The sphere was generated by direct transfer of undifferentiated ES cells into serum free medium and propagation of the resulting spheres for 5 passages. (Scale bar 25 microns).

FIG. 13 shows indirect immunofluorescence staining of a spheres 4 hours after plating on adhesive substrate for the intermediate filament nestin. Cells at the base of the sphere were placed in plane of focus to illustrate filamentous staining. The sphere was generated by direct transfer of undifferentiated ES cells into serum free medium and propagation of resulting spheres for 5 passages. (Scale bar 25 microns).

FIG. 14 shows indirect immunofluorescence microscopy of a differentiated cell decorated with antibodies against the oligodendrocyte progenitor

marker 04. (Scale bar 12.5 microns).

FIG. 15 shows indirect immunofluorescence staining of a sphere 4 hours after plating on adhesive substrate for the intermediate filament vimentin. Cells at the base of the sphere were placed in plane of focus to illustrate filamentous staining. The sphere was generated by direct transfer of undifferentiated ES cells into serum free medium and propagation of resulting spheres for 7 passages. (Scale bar 25 microns). FIG. 16 shows the growth pattern of spheres that were generated directly from undifferentiated ES cells. Each bar represents the mean (+-SD) increment in volume per week of 24 spheres at first to sixteen weeks after derivation. A more excessive growth rate is evident during the first 5 weeks.

FIG. 17 shows persistent growth in the volume of spheres along time. Each bar represents the mean (+-SD) increment in volume per week of 24 spheres at nine to twenty one weeks after derivation. The spheres were generated

from differentiating ES colonies.

FIG. 18 shows linear correlation between the volume of spheres and the number of progenitor cells within a sphere. Spheres of various diameters, that were generated directly from undifferentiated ES cells and were propagated 5-7 weeks, were dissaggregated into single cell suspension and

the number of cells per sphere was counted.

FIG. 19 shows RT-PCR analysis of gene expression in ES cells (a week after passage) and neural spheres derived from differentiating colonies and directly from undifferentiated ES cell. All panels show 2% agarose gels stained with ethidium bromide. Lanes 1, 2 and 3, Oct-4 in ES cell culture, neural spheres derived from differentiating colonies, neural spheres derived from undifferentiated ES cells. Lane 4, stem cell culture, PCR for Oct-4 carried out with omission of reverse transcriptase. Lanes 5, 6, and 7, nestin in ES cell culture, neural spheres derived from undifferentiated ES cells. Lane 8, stem cell culture, PCR for nestin carried out with omission of reverse transcriptase. Lanes 9, 10 and 11, Pax-6 in ES cell culture, neural spheres derived from differentiating colonies, neural spheres derived from undifferentiated ES cells. Lane 12, stem cell culture, PCR for Pax-6 carried out with omission of reverse transcriptase. Lane 13, 100 bp DNA ladder. Oct-4 band is 320 bp, nestin is 208 bp and Pax-6 is 274 bp.

FIG. 20 shows indirect immunofluorescence microscopy of differentiated ***astrocyte*** cells decorated with antibody against GFAP. (Scale bar

25 microns).

FIG. 21 shows indirect immunofluorescence microscopy of brain sections of two mice (A and B) 4 weeks after transplantation of human neural precursors prelabeled with BrDU. Cells with a nucleus decorated with anti BrDU (brown stain, black arrow) are evident near the ventricular surface (white arrow indicate mouse unstained puels).

(white arrow indicate mouse unstained nuclei, bar=20 microns).

FIG. 22 shows indirect immunofluorescence microscopy of brain sections of a mice 4 weeks after transplantation of human neural precursors prelabeled with BrDU. Wide spread distribution of transplanted human cells decorated by anti BrDU antibodies is evident in the periventricular areas. The periventricular area in A is demonstrated at a higher magnification in B and C. (Bars=150, 60 and 30 microns in A, B and C). FIG. 23 shows indirect immunocytochemical microscopy of brain sections of

FIG. 23 shows indirect immunocytochemical microscopy of brain sections of a mice 4 weeks after transplantation of human neural precursors prelabeled with BrDU. The transplanted human cells are migrating along

the rostral migratory stream (bar=150 microns).

FIG. 24 shows RT-PCR analysis of gene expression in neural spheres derived from differentiating (A) and undifferentiated (B) ES cells. All panels show 2% agarose gels stained with ethidium bromide. Lanes 1 and 10, 100 bpDNA ladder; Lane 2, CD34; Lane 3, Flk-1; lane4, HNF-3; lane 5, alfafetoprotein. Lanes 6-9 PCR reaction on the same samples as lanes 2-5 carried out with the omission of reverse transcriptase. CD-34 band is 200

bp, Flk-1 is 199, HNF-3 is 390, AFP is 340 bp.
FIG. 25 shows by RT-PCR analysis the expression of GFAP and the pip gene in differentiated cells from neural spheres derived from differentiating ES cell colonies. The expression of GFAP indicates differentiation into ***astrocytes*** while the presence of both dm-20 and pip transcripts indicate that differentiation into oligodendrocyte cells has occurred. Lanes 2, 4, 6 and lanes 3, 5, 7 are from two separate RNA samples from differentiated spheres that were independently derived from ES cells. Lane 1 and 8, 100 bp DNA ladder; Lanes 2 and 4, GFAP; lanes 3 and 5, plp and dm-20; lanes 6 and 7, PCR reaction on the same samples as lanes 3 and

5 carried out with the omission of reverse transcriptase. GFAP band is 383, pip band is 354 bp and dm-20 is 249 bp.
FIG. 26 shows a dark field stereomicroscopic photograph of areas (arrows) destined to give rise to neural precursors in a differentiating ES cell

colony 3 weeks after passage (bar=1.6 mm).

FIG. 27 shows indirect immunochemical analysis of marker expression in cultures of neurons derived from progenitor cells that were derived directly from undifferentiated ES cells: A, indirect immunofluorescence microscopy of neurits decorated with antibody against 160 kDa neurofilament protein. B and C, indirect immunofluorescence staining of differentiated cells for MAP2a+b and beta-tubulin III. Scale bars: Ā 100 microns, B and C 10 microns.

FIG. 28 shows indirect immunochemical analysis of the expression of tyrosine hydroxylase. Neurits (A) and a differentiated cell (B) are decorated with antibodies against tyrosine hydroxylase. Scale bars: 30

astrocyte FIG. 29 shows in vivo differentiation into transplanted human neural progenitors prelabeled with BrDU. Donor cells are identified by indirect immunochemical detection of BrDU (dark nuclei, arrows). Duel staining demonstrates donor cells decorated by anti GFAP (orange). Transplanted cells are migrating into the brain parenchyma (white arrow) and are also found in the periventricular zone (dark arrow) (A), A higher magnification of cells that have differentiated into ***astrocytes*** and migrated into the host brain (B).

FIG. 30 shows in vivo differentiation into oligodendrocyte cells of transplanted human neural progenitors prelabeled with BrDU. Donor cells are identified by indirect immunochemical detection of BrDU (dark nuclei, arrows). Duel staining demonstrates donor cells decorated by anti CNPase

(orange).

FIG. 31 shows cumulative growth curve for human neural progenitors derived from differentiating colonies. (A) Continuous growth is evident during an 18-22 week period. The increment in the volume of the spheres was continuously monitored as an indirect measure of the increase in cell numbers. A linear positive correlation between the volume of the spheres and the number of cells within the spheres (B, insert) was maintained along cultivation. It supported the validity of monitoring the increment of sphere volume as an indirect indicator of cell proliferation. FIG. 32 shows RT-PCR analysis of the expression of non-neural markers in

human ES derived spheres. All panels show 2% agarose gels stained with ethidium bromide. The symbols + and indicate whether the PCR reaction was performed with or without the addition of reverse transcriptase. A 1 Kb plus DNA ladder was used in all panels. beta-actin band is 291 bp, keratin is 780 bp, Flk-1 is 199 bp, CD34 is 200 bp, AC-133 is 200 bp, transferin is 367 bp, amylase is 490 bp and alpha 1 anti trypsin is 360

33 shows a phase contrast micrograph of differentiated cells growing out from a sphere 2 weeks after plating onto an adhesive surface and

culture in the absence of growth factors. Scale bar is 200 mu m.

FIG. 34 shows RT-PCR analysis of the expression of neuronal and glial markers in differentiated cells originating from human ES derived neural spheres. All panels show 2% agarose gels stained with ethidium bromide. The symbols + and -indicate whether the PCR reaction was performed with or without the addition of reverse transcriptase. A 1 Kb plus DNA ladder was used in all panels. Plp and dm-20 bands are 354 bp and 249 bp respectively, MBP is 379 bp, GFAP is 383 bp, NSE is 254 bp and NF-M is 430 bp.

FIG. 35 shows indirect immunochemical analysis of the expression of serotonin (A) and GABA (B). Scale bars are 20 mu m.

FIG. 36 shows dissemination of transplanted BrdU+ human ESderived neural

progenitor cells in the mouse host brain.

(A) At 2 days after transplantation most cells were found lining the ventricular wall. (B) After 4-6 weeks most cells had left the ventricles (V) and populated the corpus callosum (CC), fimbria (fim), internal capsule (i.c.). BrdU+ cells were not found in the striatum (str) or CA region of the hippocampus (hipp). (C) Chains of BrdU+ cells were found in the rostral migratory stream (RMS). (D) BrdU+ cells in the

periventricular white matter. (E) Higher magnification of D, to show nuclear specific localization of BrdU. FIG. 37 shows identification of the transplanted cells in the brain by human and neural-lineage specific markers. (A) A typical chain of transplanted cells in the corpus callosum, stained with human specific anti-mitochondrial antibody. The mitochondrial staining (green fluorescence) on Nomarsky background (blue, cell nuclei indicated by asterisk) shows a typical perinuclear localization. (B) Double staining for BrdU (green fluorescence) and human specific anti ribonuclear protein (red fluorescence) shows nuclear co-localization, indicating that BrdU+cells were indeed of human origin. (C) A GFAP+ ***astrocyte*** (red) from the periventricular region, colabeled with BrdU (green), indicating its origin from the graft. (D) An NG2+ oligodendrocyte progenitor (red) in the periventricular region, co-labeled with BrdU (green). (E) A CNPase+ oligodendrocyte (red) in the corpus callosum, colabeled with BrdU (immunohistochemistry, shown as dark nucleus in Nomarsky). (F) Neuronal (immunohistochemistry, shown as dark nucleus in Nomarsky). (F) Neuronal processes in the fimbria, stained with a human specific anti-70 kDa neurofilament. (G) A beta III-tubulin+ neuron (green fluorescence) in the olfactory bulb, co-labeled with BrdU (as dark nucleus (arrow) in Nomarsky). Bars=10 mu m. ! ANSWER 29 OF 55 IFIPAT COPYRIGHT 2004 IFI on STN DUPLICATE 8 10124433 IFIPAT; IFIUDB; IFICDB EMBRYONIC STEM CELLS AND NEURAL PROGENITOR CELLS DERIVED THEREFROM; SUCH AS NEURAL PROGENITOR CELLS CAPABLE OF GIVING RISE TO MATURE SOMATIC CELLS INCLUDING NEURAL CELLS AND/OR GLIAL CELLS RECOGNIZABLE BY EXPRESSION OF SPECIFIC MARKERS Ben-Hur Tamir (IL); Pera Martin Frederick (AU); Reubinoff Benjamin Eithan (IL) Unassigned Or Assigned To Individual (68000) A1 20020606 US 2002068045 US 2001-808382 20010314 AI AU 2000-6211 20000314 AU 2000-1279 20001106 AU 2001-2920 20010206 US 2002068045 20020606 Utility; Patent Application - First Publication CHEMICAL **APPLICATION** MN 85 30 Figure(s). IG. 1 shows phase contrast micrographs of ES cells and their differentiated progeny. A, inner cell mass three days after plating. B, colony of ES cells. C, higher magnification of an area of an ES cell colony. D, an area of an ES cell colony. The colony of the days of the process of the colony of the colony of the colony. differentiation during routine passage. E, a colony four days after plating in the absence of a feeder cell layer but in the presence of 2000 units/ml human LIF undergoing differentiation in its periphery, F, neuronal cells in a high density culture. Scale bars: A and C, 25 microns; B and E, 100 microns; D and F, 50 microns. FIG. 2 shows marker expression in ES cells and their differentiated somatic progeny. A, ES cell colony showing histochemical staining for alkaline phosphatase. B. ES cell colony stained with antibody MC-813-70 recognising the SSEA-4 epitope. C, ES cell colony stained with antibody TRA1-60. D, ES cell colony stained with antibody GCTM-2. E, high density culture, cell body and processes of a cell stained with antineurofilament 68 kDa protein. F, high density culture, cluster of cells and network of processes emanating from them stained with antibody against neural cell adhesion molecule. G, high density culture, cells showing cytoplasmic filaments stained with antibody to muscle actin. H, high density culture, cell showing cytoplasmic filaments stained with antibody to desmin. Scale bars: A, 100 microns; B-D, and F, 200 microns; E, G and H, 50 microns. FIG. 3 shows RT-PCR analysis of gene expression in Es cells and their differentiated derivatives. All panels show 1.5% agarose gels stained with ethidium bromide. A, expression of Oct-4 and b-actin in Es stem cells and high density cultures. Lane 1, 100 bpDNA ladder. Lane 2, stem cell culture, b-actin. Lane 3, stem cell culture, Oct-4. Lane 4, stem cell culture. cell culture, PCR for Oct-4 carried out with omission of reverse transcriptase. Lane 5, high density culture, b-actin. Lane 6, high density culture, Oct-4. Lane 7, high density culture, PCR for Oct-4 carried out with omission of reverse transcriptase. b-actin band is 200 bp and Oct-4 band is 320 bp. B, expression of nestin and Pax-6 in neural progenitor cells that were derived from differentiating ES colonies. Left lane, 100 bp DNA ladder; lane 1, b-actin in HX 142 neuroblastoma cell line (positive control for nestin PCR); lane 2, b-actin in neural progenitor cells; lane 3, nestin in HX 142 neuroblastoma cell line; lane

4, nestin in neural progenitor cells; lane 5, nestin PCR on same sample as lane 4 without addition of reverse transcriptase; lane 6, Pax-6; lane 7, Pax-6 PCR on same sample as line 6 without addition of reverse transcriptase. Nestin band is 208 bp, Pax-6 is 274 bp. C, expression of glutamic acid decarboxylase in cultures of neurons. Left lane, 100 bp DNA ladder; lane 1, b-actin; lane 2, b-actin PCR on same sample as lane 1 without addition of reverse transcriptase; lane 3, elutamic acid without addition of reverse transcriptase; lane 3, glutamic acid decarboxylase; lane 4 glutamic acid decarboxylase on same sample as lane 3 without addition of reverse transcriptase. Glutamic acid decarboxylase band is 284 bp. D, expression of GABA A alpha 2 receptor. Left lane, 100 bp DNA ladder; lane 1, b-actin; lane 2, GABA A alpha 2 receptor; lane 3, PCR without addition of reverse transcriptase. GABA A alpha 2 receptor subunit band is 471 bp.

FIG. 4 shows histology of differentiated elements found in teratomas formed in the testis of SCID mice following inoculation of HES-1 or HES-2 colonies. A, cartilage and squamous epithelium, HES-2. B, neural rosettes, HES-2. C, ganglion, gland and striated muscle, HES-1. D, bone and cartilage, HES-1. E, glandular epithelium, HES-1. F, ciliated columnar epithelium, HEŚ-I. Scale bars: A-E, 100 microns; F, 50 microns. FIG. 5 shows phase contrast microscopy and immunochemical analysis of

marker expression in neural progenitor cells isolated from differentiating ES cultures. A, phase contrast image of a sphere formed in serum-free medium. B-D, indirect immunofluorescence staining of spheres, 4 hours after plating on adhesive substrate, for N-CAM, nestin, and vimentin respectively. In C and D, cells at the base of the sphere were placed in plane of focus to illustrate filamentous staining; confocal examination revealed that cells throughout the sphere were decorated by both antibodies. Scale bar is 100 microns in all panels.

FIG. 6 shows phase contrast appearance and marker expression in cultures of neurons derived from progenitor cells shown in FIG. 5. A, phase contrast micrograph of differentiated cells emanating from a sphere plated onto adhesive surface. B-H, indirect immunofluorescence microscopy of differentiated cells decorated with antibodies against 200 kDa neruofilament protein (B), 160 kDa neurofilament protein (C), MAP2a+b (D), glutamate (E), synaptophysin (F), glutamic acid decarboxylase (G) and beta-tubulin (H). Scale bars: A,;B, 100 microns; C, 200 mircons; D, 20 microns; E and F, 10 microns; G, 20 microns; H, 25 microns.

FIG. 7 shows neural precursors proliferating as a monolayer on a plastic

tissue culture dish in the presence of EGF and bFGF. These monolayer cultures of proliferating cells were obtained after prolonged cultivation (2-3 weeks) of the spheres in the presence of growth factors without sub-culturing.

FIG. 8 shows phase contrast appearance of a culture consisting of differentiated neural cells.

FIG. 9 shows phase contrast appearance of a sphere that is formed 72 hours after the transfer of a clump of undifferentiated ES cells into serum free medium (Scale bar 100 microns).

FIG. 10 shows linear correlation between the volume of spheres and the number of progenitor cells within a sphere. Spheres of various diameters that were generated from differentiating ES colonies and were propagated for 14-15 weeks were dissaggregated into single cell suspension and the number of cells per sphere was counted.

FIG. 11 shows indirect immunofluorescence staining of a sphere, 4 hours after plating on adhesive substrate, for N-CAM. The sphere was generated by direct transfer of undifferentiated ES cells into serum free medium and propagation of the resulting spheres for 5 passages. (Scale bar 100

FIG. 12 shows indirect immunofluorescence membraneous staining for N-CAM of single cells at the periphery of a sphere 4 hours after plating on adhesive substrate. The sphere was generated by direct transfer of undifferentiated ES cells into serum free medium and propagation of the

resulting spheres for 5 passages. (Scale bar 25 microns). FIG. 13 shows indirect immunofluorescence staining of a spheres 4 hours after plating on adhesive substrate for the intermediate filament nestin. Cells at the base of the sphere were placed in plane of focus to illustrate filamentous staining. The sphere was generated by direct transfer of undifferentiated ES cells into serum free medium and propagation of resulting spheres for 5 passages. (Scale bar 25 microns). FIG. 14 shows indirect immunofluorescence microscopy of a differentiated cell decorated with antibodies against the oligodendrocyte progenitor

marker 04. (Scale bar 12.5 microns).
FIG. 15 shows indirect immunofluorescence staining of a sphere 4 hours after plating on adhesive substrate for the intermediate filament vimentin. Cells at the base of the sphere were placed in plane of focus to illustrate filamentous staining. The sphere was generated by direct transfer of undifferentiated ES cells into serum free medium and

propagation of resulting spheres for 7 passages. (Scale bar 25 microns). FIG. 16 shows the growth pattern of spheres that were generated directly from undifferentiated ES cells. Each bar represents the mean (+-SD) increment in volume per week of 24 spheres at first to twelve weeks after derivation. A more excessive growth rate is evident during the first 5

FIG. 17 shows persistent growth in the volume of spheres along time. Each bar represents the mean (+-SD) increment in volume per week of 24 spheres at nine to twenty one weeks after derivation. The spheres were generated

from differentiating ES colonies.

FIG. 18 shows linear correlation between the volume of spheres and the number of progenitor cells within a sphere. Spheres of various diameters, that were generated directly from undifferentiated ES cells and were propagated 5-7 weeks, were dissaggregated into single cell suspension and

the number of cells per sphere was counted.

FIG. 19 shows RT-PCR analysis of gene expression in ES cells (a week after passage) and neural spheres derived from differentiating colonies and directly from undifferentiated ES cell. All panels show 2% agarose gels stained with ethidium bromide. Lanes 1, 2 and 3, Oct-4 in ES cell culture, neural spheres derived from differentiating colonies, neural spheres derived from undifferentiated ES cells. Lane 4, stem cell culture, PCR for Oct-4 carried out with omission of reverse transcriptase. Lanes 5, 6, and 7, nestin in ES cell culture, neural spheres derived from differentiating colonies, neural spheres derived from undifferentiated ES cells. Lane 8, stem cell culture, PCR for nestin carried out with omission of reverse transcriptase. Lanes 9, 10 and 11, Pax-6 in ES cell culture, neural spheres derived from differentiating colonies, neural spheres derived from undifferentiated ES cells. Lane 12, stem cell culture, PCR for Pax-6 carried out with omission of reverse transcriptase. Lane 13, 100 bp DNA ladder. Oct-4 band is 320 bp, nestin is 208 bp and Pax-6 is 274 bp.

FIG. 20 shows indirect immunofluorescence microscopy of differentiated ***astrocyte*** cells decorated with antibody against GFAP. (Scale bar

25 microns).

FIG. 21 shows indirect immunofluorescence microscopy of brain sections of two mice (A and B) 4 weeks after transplantation of human neural precursors prelabeled with BrDU. Cells with a nucleus decorated with anti BrDU (brown stain, black arrow) are evident near the ventricular surface (white arrow indicate mouse unstained nuclei, bar=20 microns).

FIG. 22 shows indirect immunofluorescence microscopy of brain sections of a mice 4 weeks after transplantation of human neural precursors prelabeled with BrDU. Wide spread distribution of transplanted human cells decorated by anti BrDU antibodies is evident in the periventricular areas. The periventricular area in A is demonstrated at a higher magnification in B and C. (Bars=150, 60 and 30 microns in A, B and C). FIG. 23 shows indirect immunocytochemical microscopy of brain sections of

a mice 4 weeks after transplantation of human neural precursors prelabeled with BrDU. The transplanted human cells are migrating along the rostral migratory stream (bar=150 microns).

FIG. 24 shows RT-PCR analysis of gene expression in neural spheres derived from differentiating (A) and undifferentiated (B) ES cells. All panels show 2% agarose gels stained with ethidium bromide. Lanes 1 and 10, 100 bpDNA ladder; Lane 2, CD34; Lane 3, Flk-1; lane 4, HNF-3; lane 5, alfafetoprotein. Lanes 6-9 PCR reaction on the same samples as lanes 2-5 carried out with the omission of reverse transcriptase. CD-34 band is 200

bp, Flk-1 is 199, HNF-3 is 390, AFP is 340 bp. FIG. 25 shows by RT-PCR analysis the expression of GFAP and the plp gene in differentiated cells from neural spheres derived from differentiating ES cell colonies. The expression of GFAP indicates differentiation into "***astrocytes*** while the presence of both dm-20 and plp transcript while the presence of both dm-20 and plp transcripts indicate that differentiation into oligodendrocyte cells has occurred. Lanes 2,4,6 and lanes 3,5,7 are from two separate RNA samples from differentiated spheres that were independently derived from ES cells. Lane 1 and 8, 100 bp DNA ladder; Lanes 2 and 4, GFAP; lanes 3 and 5, plp and dm-20; lanes 6 and 7, PCR reaction on the same samples as lanes 3 and 5 carried out with the omission of reverse transcriptase. GFAP band is 383, plp band is 354 bp and dm-20 is 249 bp.

FIG. 26 shows a dark field stereomicroscopic photograph of areas (arrows) destined to give rise to neural precursors in a differentiating ES cell

colony 3 weeks after passage (bar=1.6 mm).

FIG. 27 shows indirect immunochemical analysis of marker expression in cultures of neurons derived from progenitor cells that were derived directly from undifferentiated ES cells: A, indirect immunofluorescence microscopy of neurits decorated with antibody against 160 kDa neruofilament protein. B and C, indirect immunofluorescence staining of differentiated cells for MAP2a+b and beta-tubulin III. Scale bars: A 100

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microns, B and C 10 microns.
     FIG. 28 shows indirect immunochemical analysis of the expression of
      tyrosine hydroxylase. Neurits (A) and a differentiated cell (B) are
      decorated with antibodies against tyrosine hydroxylase. Scale bars: 30
     FIG. 29 shows in vivo differentiation into
                                                      ***astrocyte***
      transplanted human neural progenitors prelabeled with BrDU. Donor cells
      are identified by indirect immunochemical detection of BrDU (dark nuclei,
      arrows). Duel staining demonstrates donor cells decorated by anti GFAP
      (orange). Transplanted cells are migrating into the brain parenchyma
       (white arrow) and are also found in the periventricular zone (dark arrow)
      (A), A higher magnification of cells that have differentiated into ***astrocytes*** and migrated into the host brain (B).
     ***astrocytes*** and migrated into the host brain (B).
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      transplanted human neural progenitors prelabeled with BrDU. Donor cells
      are identified by indirect immunochemical detection of BrDU (dark nuclei,
      arrows). Duel staining demonstrates donor cells decorated by anti CNPase
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       Thomas, Melissa K., Boston, MA, UNITED STATES
       Abraham, Elizabeth J., Quincy, MA, UNITED STATES
       Vallejo, Mario, Madrid, SPAIN
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       ICM: A61K048-00
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       INCLS: 435/001.100; 435/174.000; 435/177.000; 435/325.000; 435/366.000;
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       NCLS:
               435/001.100; 435/174.000; 435/177.000; 435/325.000; 435/366.000;
               435/395.000
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       ICM: C12N005-06
       ICS: C12N005-08; C12N011-00; C12N011-02
       424/93.7; 435/174; 435/325; 435/395; 435/366; 435/177; 435/1.1
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    Department of Anatomy and Neurobiology, Washington University School of
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    Radial Glial Cells as Neuronal Precursors: The Next Generation?
    Gregg, C.T.; Chojnacki, A.K.; Weiss, S.*
HSC 2164-3330 Hospital Dr. NW, Calgary, AB, T2N 4N1, Canada; E-mail:
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    no. 6, pp. 708-713. Special issue: Stem cells..
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    Division of Paediatrics, Obstetrics and Gynaecology, Imperial College of
    Science, Technology and Medicine, Du Cane Road, Hammersmith Hospital Campus, London, W12 ONN, UK
    h.mehmet@ic.ac.uk
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    CODEN: JPTLAS. ISSN: 0022-3417.
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    Entered STN: 21 Aug 2002
    Last Updated on STN: 21 Aug 2002
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              PubMed ID: 11921204
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    Medicine, Salt Lake City, Utah, USA.
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Journal code: 8806785. ISSN: 0894-1491.
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    MEDLINE 2002187447
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    200205
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    Entered STN: 20020726
    Last Updated on STN: 20020726
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    Alternative sources of neurons and glia from somatic stem cells
Torrente Y.; Belicchi M.; Pisati F.; Pagano S.F.; Fortunato F.; Sironi
M.; Grazia D'Angelo M.; Parati E.A.; Scarlato G.; Bresolin N.
Prof. N. Bresolin, Institute of Clinical Neurology, University of Milan,
    Ospedale Policlinico, via Francesco Sforza 35, 20122 Milan, Italy.
     E-mail: radponti@unimi.it
    Cell Transplantation, (2002), 11/1 (25-34), 35 reference(s) CODEN: CTRAE8 ISSN: 0963-6897
     Journal; Article
     United States
     English
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      ***Transdifferentiation***
                                        of Human Haemopoietic Lineage Negative Bone
   Marrow Cells to Neural Cells by Cytokines and Chemical Inducing Agents.
   Tao, Helen [Reprint Author]; Rao, Renuka S. [Reprint Author]; Ma, David D.
   F. [Reprint Author]
   Department of Haematology and Haematopoietic Stem Cell Transplantation, St
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Blood, (November 16 2002) Vol. 100, No. 11, pp. Abstract No. 4123. print.
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Philadelphia, PA, USA. December 06-10, 2002. American Society of
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   Conference; (Meeting)
   Conference; Abstract; (Meeting Abstract)
   Conference; (Meeting Poster)
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   Entered STN: 13 Aug 2003
   Last Updated on STN: 13 Aug 2003
     ANSWER 38 OF 55 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
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     2002-06780 BIOTECHDS
In vitro ***transdifferentiation***
                                                    of mammalian cells from glial
                                                           ***astrocytes***
     cell type to neurons, oligodendrocytes and
     comprises culturing the cells to form group of cells and exposing the
     cells to a growth factor;
        human fetal and adult mammal
                                              ***astrocyte***
                                                                   and stem cell
        transactivation in a culture vessel for the production of multipotent
        cell for xenotransplantation, Alzheimer disease, Parkinson disease,
        stroke recovery, brain, spinalcord damage therapy
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      Thomas, Melissa K., Boston, MA, United States
      Vallejo, Mario, Madrid, Spain
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RAI
                              20000628 (60)
      US 2000-215109P
      US 2000-238880P
                              20001006 (60)
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1.CNT 2114
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      INCLM: 424/093.210
      INCLS: 514/009.000; 424/152.100; 435/366.000
NCLM: 424/093.210
      NCLS: 514/009.000; 424/152.100; 435/366.000
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ICM: A61K048-00
      ICS: C12N005-08; A61K039-395
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      Moss, Peter Ian, London, Great Britain
      Walters, David Martin, London, Great Britain
Pointer, Graham, London, Great Britain
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      Utility
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              424/093.700
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      Fung, Brenda, Belmont, MA, United States
Pang, Kevin, Belmont, MA, United States
Kagan, David, Brighton, MA, United States
      Curis, Inc., Cambridge, MA, United States (U.S. corporation)
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      NCLM:
      NCLS:
              435/325.000; 435/371.000; 435/378.000
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      ICM: C12N005-02
      ICS: C12N005-00; C12N005-08
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   B04 D16
   HUR-BEN, T; PERA, M F; REUBINOFF, B E; BEN-HUR, T
    (HADA-N) HADASIT MEDICAL RES SERVICES & DEV; (REUB-I) REUBINOFF B E; (MONU) UNIV MONASH; (UYSI-N) UNIV SINGAPORE NAT; (ESCE-N) ES CELL INT PTE
   LTD; (BENH-I) BEN-HUR T; (PERA-I) PERA M F; (REUB-N) REUBINOFF
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            DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC
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ICS A61K035-28; A61K035-30; A61K048-00; A61P009-00; A61P017-02; A61P025-00; A61P025-28; A61P037-00; A61P043-00; C12N005-10
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     Univ Milan, Inst Clin Neurol, Osped Policlin, Padigl Ponti, Via Francesco
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CS
     Institut Leon Fredericq, Universite de Liege, Place Delcour 17, B 4020,
     Liege, Belgium
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       Sakaguchi D.S.; Janick L.M.; Reh T.A.
D.S. Sakaguchi, Department of Zoology and Genetics, 339 Science II, Iowa
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CS
       State University, Ames, IA 50011, United States.
E-mail: dssakagu@iastate.edu
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       Hayashi Y.; Nomuha M.; Yamagishi S.-I.; Harada S.-I.; Yamashita J.;
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